THYROID SODIUM/IODIDE SYMPORTER AND NUCLEIC ACID ENCODING SAME

Statement of Government Interest

This invention was made with government support under NIH Grant No. DK-41544. As such, the government has certain rights in this invention.

Background of the Invention

The principal function of the thyroid gland is to produce the hormones thyroxine (tetraiodothyronine, T_4) and tri-iodothyronine (T_3), both of which play essential roles in regulating intermediary metabolism in virtually all tissues and in maturation of the nervous system, skeletal muscle and lungs in the developing fetus and the newborn (Werner and Ingbar, The Thyroid: A fundamental and clinical text (Braverman and Utiger, eds.) (1991) pp. 1-1362, Lippincott, Philadelphia; DeGroot, Endocrinology (DeGroot, ed.) (1995) Grune and Stratton, Orlando, Florida). Thyroxine and T_3 are unique hormones in that both contain iodine as an essential constituent.

The hormone-producing thyroid follicular cells or thyrocytes display a highly specialized ability to transport iodide, the anionic form of iodine. This ability is an apparent cellular adaptation to sequester environmentally scarce iodine, thus ensuring adequate thyroid hormone production in most cases. Nevertheless, insufficient dietary supply of iodine is still prevalent among millions of people in many regions of the world, leading to endemic iodine deficiency disorders (IDD) often associated with lower-than-normal thyroid hormone production (Medeiros-Neto, et al., Thyroid Research, (Robbins and Braverman, eds.), (1976) p.497, Excerpta Medica, Amsterdam). The translocation of iodide into the thyroid for thyroid hormogenesis involves two separate processes: iodide accumulation and iodide efflux.

Iodide accumulation is the translocation of iodide from the interstitium into the follicular cells across the basolateral plasma membrane. Iodide

accumulation is a Na^+-dependent active transport process catalyzed by the sodium/iodide symporter, an intrinsic plasma membrane protein located in the basolateral end of thyrocytes that couples the energy released by the inward "downhill" translocation of Na^+ down its electrochemical gradient to driving the simultaneous inward "uphill" translocation of iodide against its electrochemical gradient (Carrasco, Biochim. Biophys. Acta. 1154:65-82 (1993)). The Na^+ gradient acting as a driving force for iodide accumulation is generated by the ouabain-sensitive, K^+_{out}-activated Na^+/K^+ ATPase. Thus, Na^+-dependent iodide accumulation (i.e. sodium/iodide symport activity) is the first and rate-limiting step in the biosynthesis of thyroid hormones. Sodium/iodide symport activity in the thyroid is characteristically blocked by the competitive inhibitor perchlorate.

Iodide efflux is the transfer of iodide from the cytoplasm of thyrocytes towards the colloid across the apical plasma membrane. Iodide efflux is a passive diffusion mechanism that has been proposed to be mediated by an iodide channel located in the apical membrane of thyrocytes (Nilsson, et al., Acta Endocrinol 126:67-74 (1992); Golstein, et al., Am. J. Physiol. 263:C590-C5975 (1992)). The colloid, where the large hormone precursor thyroglobulin (Tg) is stored, is located in the follicular lumen, an extracellular compartment. Iodide is ultimately required at the cell/colloid interface because this is the site where, to a large extent, hormone biosynthesis takes place (Werner and Ingbar, supra; Degroot, supra). Accumulated iodide that has reached the cell/colloid interface is oxidized and incorporated into some tyrosyl residues within the Tg molecule in a reaction catalyzed by thyroid peroxidase (TPO), leading to the subsequent coupling of iodotyrosine residues. This incorporation of iodide into organic molecules is called "iodide organification", a reaction pharmacologically blocked by such anti-thyroid agents as 6-n-propyl-2-thiouracil (PTU).

All steps in the thyroid hormone biosynthetic pathway are stimulated by thyroid stimulating hormone (TSH) secreted from the pituitary. The effect of TSH results from binding of the hormone to the TSH receptor, which is also located in the basolateral membrane of the follicular cells.

Sodium/iodide symporter confers to the thyroid gland its most readily distinctive functional attribute, i.e. its ability to actively accumulate iodine. Sodium/iodide symporter provides the molecular basis for the thyroidal radioiodide uptake test and for thyroid scintigraphy, two thyroid function tests of considerable value as diagnostic aids in a variety of thyroid pathological conditions (Werner and Ingbar, supra; Degroot, supra). For example, the possible existence of thyroid cancer must be ruled out whenever a thyroid nodule is detected. Thyroid nodules that are determined by scintigraphy to accumulate iodine equally or more efficiently than the normal surrounding tissue are generally benign, while most thyroid cancers display markedly reduced iodine accumulation activity relative to healthy tissue. Still, sodium/iodide symporter is sufficiently active in some thyroid cancers and metastases to render them amenable to treatment with radioiodine (Werner and Ingbar, supra; Degroot, supra). Conversely, large doses of radiation reaching the gland via sodium/iodide symporter in the form of iodine isotopes can cause thyroid cancer. The most dramatic example of this is the alarming rise in the incidence of thyroid cancer cases in Ukraine and Belarus in the wake of the 1986 Chernobyl power plant accident (Likhtarev, et al., Nature 375:365 (1995)). In this instance, ^{131}I in the nuclear fallout was ingested largely through milk, mostly by young children, and concentrated in the thyroid via sodium/iodide symporter. Among major thyroid proteins involved in hormogenesis, the TSH receptor, Tg and TPO have all been characterized in considerable molecular

detail (Parmentier, et al., Science 246:1620-1622 (1989); Mercken, et al., Nature 316:647-651 (1985); Magnusson, et al., J. Biol. Chem. 262:13885-13888 (1987)). Prior to the present invention however, the cDNA encoding sodium/iodide symporter has not been cloned or characterized.

Summary of the Invention

The present invention provides a purified and isolated nucleic acid encoding sodium/iodide symporter, a vector comprising this nucleic acid and a host cell transformed by this vector. Also provided by the present invention is a nucleic acid probe which hybridizes to nucleic acid encoding sodium/iodide symporter, a mixture of nucleic acid probes each of which hybridizes to nucleic acid encoding sodium/iodide symporter and a kit comprising one or more nucleic acid probes which hybridize to nucleic acid encoding sodium/iodide symporter.

The present invention also provides a method for producing recombinant sodium/iodide symporter comprising growing a host cell transformed with a vector comprising nucleic acid encoding sodium/iodide symporter in culture and recovering sodium/iodide symporter from the culture.

The present invention further provides a purified sodium/iodide symporter or an analogue thereof, an antibody immunoreactive with sodium/iodide symporter or an analogue thereof, and a kit comprising an antibody immunoreactive with sodium/iodide symporter.

The present invention also provides a method for diagnosing a thyroid disorder in a subject comprising detecting one or more mutations in nucleic acid encoding sodium/iodide symporter, a mutated sodium/iodide symporter, or a decreased concentration of sodium/iodide symporter in the subject relative to normal physiological levels of the sodium/iodide symporter.

In addition, the present invention provides a method for treating a thyroid disorder caused by a mutated nucleic acid encoding sodium/iodide symporter comprising

introducing nucleic acid encoding sodium/iodide symporter into substantially all of the thyroid cells of the subject such that an amount of the sodium/iodide symporter effective to treat the thyroid disorder is expressed in the thyroid cells.

The present invention also provides a recombinant viral vector capable of introducing nucleic acid encoding sodium/iodide symporter into a target cell such that the target cell expresses sodium/iodide symporter, the vector comprising (a) nucleic acid of or corresponding to at least a portion of the genome of a virus, the portion being capable of infecting the target cell, and (b) nucleic acid encoding a sodium/iodide symporter operably linked to the viral nucleic acid.

The present invention further provides a method for selectively ablating a target tissue in a subject comprising: (a) introducing nucleic acid encoding sodium/iodide symporter into substantially all cells of the target tissue such that the cells exhibit sodium/iodide symport activity; and (b) supplying radioactive iodide to the cells of the target tissue in an amount sufficient to ablate the target tissue upon uptake of the radioactive iodide by the cells.

The present invention also provides a method for identifying an iodide transport protein from non-thyroid tissue comprising contacting nucleic acid from the non-thyroid tissue with a nucleic acid probe made from nucleic acid encoding sodium/iodide symporter and detecting hybridization thereof.

Finally, the present invention provides a non-human, transgenic animal model for a thyroid disorder comprising mutated nucleic acid encoding sodium/iodide symporter incorporated into thyroid cells of the animal.

Additional objects of the invention will be apparent from the description which follows.

Brief Description of the Figures

Figure 1 is comprised of Figures 1A, 1B, 1C and 1D and depicts expression of sodium/iodide symporter in Xenopus laevis oocytes and COS cells. In Figure 1A oocytes were microinjected with water (lane 1), 50 ng mRNA from FRTL-5 cells (lane 2), or approximately 20 ng cRNA transcripts made in vitro from pools containing the indicated number of cDNA clones (lanes 3-7, numbers without parentheses at bottom of Figure 1A). Iodide accumulation was assayed in the presence of Na^+ (solid bars), absence of Na^+ (i.e. in the presence of choline, striped bars), or in the presence of both Na^+ and perchlorate (dotted bars) at 45 min, using standard methods (Weiss, et al., Endocrinology 114:1090-1098 (1984)). The number of days elapsed after microinjection when sodium/iodide (Na^+/I^-) symport activity was first detected are indicated in parentheses. Time course (Figure 1B) and kinetic analysis (Figure 1C) of iodide accumulation were assessed 60 h after oocytes were microinjected with the transcript from the sodium/iodide symporter cDNA clone. In Figure 1C, initial velocity rates of Na^+/I^- symport activity were determined at the 2 min time points at various iodide concentrations (1.25 to 250 μM). Inset: Double reciprocal plot. In Figures 1A, 1B and 1C each bar and experimental point represent the average of >5 oocytes +/- S.E. Figure 1D depicts a time course of iodide accumulation in COS cells transfected with pSV.SPORT plasmid containing sodium/iodide symporter cDNA (continuous lines) or an irrelevant insert (dotted line), or without DNA (dashed line). Iodide accumulation was assayed in the presence of Na^+ (■), absence of Na^+ (i.e. in the presence of choline, •), or in the presence of both Na^+ and perchlorate (▲ or Δ) at the indicated time points, as described elsewhere (Kaminsky, et al., Proc.Nat.Acad.Sci.USA, 91:3789-3793 (1994)). Each point represents the average of triplicate determinations +/-SE.

Sub A1 Figure 2 represents complementary nucleotide and deduced amino acid sequences of the rat sodium/iodid symporter cDNA. Nucleotides are numbered in the 5′ to 3′ direction beginning with the first base of the cloned cDNA. Untranslated sequences are in lower case and translated sequences in upper case letters. The deduced amino acid sequence (single letter code) is shown below the nucleotide sequence. The twelve putative membrane-spanning domains are shaded in grey. Three potential N-linked glycosylation sites are indicated in bold type (positions 225, 485 and 497). One putative intracellular consensus sequence for cAMP-dependent protein kinase A phosphorylation is boxed (positions 549-552). A polyadenylation signal in the 3′ untranslated domain is underlined (position 2795).

Figure 3 is comprised of Figures 3A and 3B. Figure 3A depicts a hydropathy plot of the deduced amino-acid sequence. The hydropathic analysis was based on the Kyte-Doolittle algorithm (Kyte and Doolittle, J. Mol. Biol., 157: 105-132 (1982)) with a window of 9 residues. Hydropathy values (positive correspond to hydrophobic and negative to hydrophilic regions) are plotted against amino acid position. Putative membrane-spanning domains are shown as filled-in areas under plot line. Figure 3B depicts a schematic representation of the putative topology of the rat sodium/iodide symporter in the membrane. Roman numerals indicate putative membrane-spanning domains. Potential N-linked glycosylation sites are indicated by asterisks. A putative intracellular consensus sequence for cAMP-dependent protein kinase A phosphorylation is indicated with an arrow.

Figure 4 depicts a dendrogram representing a cluster analysis of members of the Na^+-dependent cotransporter protein family. A multiple analysis was created using the PILEUP program of the Genetic Computer Group. The dendrogram is a tree representation of clustering relationships among the deduced amino acid

sequences of the cDNAs used for the analysis. The numbers in parentheses represent the percent of homology to the rat sodium/iodide symporter cDNA.

Detailed Description of the Invention

The present invention provides a purified and isolated nucleic acid encoding sodium/iodide symporter. As used herein, the nucleic acid may be genomic DNA, cDNA, RNA or antisense RNA and includes nucleic acid derived from any species, e.g., human, rat, goat, pig, mouse or cow. Due to the degeneracy of the genetic code, the nucleic acid of the present invention also includes a multitude of nucleic acid substitutions which will encode sodium/iodide symporter.

The present invention also provides a vector which comprises nucleic acid encoding sodium/iodide symporter. Such vectors may be used for storing the nucleic acid encoding sodium/iodide symporter, or for preparing multiple copies of the nucleic acid, as well as producing recombinant sodium/iodide symporter. The vectors may be constructed by inserting nucleic acid encoding sodium/iodide symporter into suitable vector nucleic acid. The term "inserted" as used herein means the ligation of a foreign DNA fragment and vector DNA by techniques such as the annealing of compatible cohesive ends generated by restriction endonuclease digestion or by use of blunt end ligation techniques. Other methods of ligating DNA molecules will be apparent to one skilled in the art.

Vectors may be derived from a number of different sources, e.g., plasmids, viral genomes, lytic bacteriophage derived from phage lambda (λ), cosmids or filamentous single-stranded bacteriophages such as M13. The nucleic acid from which the vector is derived is usually greatly reduced in size so that only those genes essential for its autonomous replication remain. The reduction in size enables the vectors to accommodate large

segments of foreign DNA. Examples of suitable vectors for storing or producing multiple copies of the nucleic acid include but are not limited to pBR322, pUC18, pUC19, pHSV-106, pJS97, pJS98, M13mp18, M13mp19, pSPORT 1, pGem, pSPORT 2, pSV•SPORT 1, pBluescript II, λZapII, λgt10, λgt11, λgt22A, and λZIPLOX.

Vectors suitable for the expression of the nucleic acid encoding sodium/iodide symporter in a host cell are well known in the art and include pET-3d (Novagen), pProEx-1 (Life Technologies), pFastBac 1 (Life Technologies), pSFV (Life Technologies), pcDNA II (Invitrogen), pSL301 (Invitrogen), pSE280 (Invitrogen), pSE380 (Invitrogen), pSE420 (Invitrogen), pTrcHis A,B,C (Invitrogen), pRSET A,B,C (Invitrogen), pYES2 (Invitrogen), pAC360 (Invitrogen), pVL1392 and pV11392 (Invitrogen), pCDM8 (Invitrogen), pcDNA I (Invitrogen), pcDNA I(amp) (Invitrogen), pZeoSV (Invitrogen), pcDNA 3 (Invitrogen), pRc/CMV (Invitrogen), pRc/RSV (Invitrogen), pREP4 (Invitrogen), pREP7 (Invitrogen), pREP8 (Invitrogen), pREP9 (Invitrogen), pREP10 (Invitrogen), pCEP4 (Invitrogen), pEBVHis (Invitrogen), and λPop6. Such vectors utilize one of a number of powerful promoters to direct the high level of expression, e.g., the lac operator-promoter or the tac promoter, metallothionine or mouse mammary tumor virus promoters.

Vectors may be introduced into host cells by a number of techniques, e.g. electroporation, DEAE dextran, cationic liposome fusion, protoplast fusion, DNA coated-microprojectile bombardment, and infection with recombinant replication-defective retroviruses. The term "transformation" denotes the introduction of a vector into a bacterial or eukaryotic host cell. As such, it encompasses transformation of bacterial cells and transfection, transduction and related methods in eukaryotic cells.

Any one of a numb r of suitable bacterial or eukaryotic host cells may be transformed with the vector of the present invention. Examples of suitable host cells are known to one skilled in the art and include but are not limited to bacterial cells such as E.coli strains c600, c600hfl, HB101, LE392, Y1090, JM103, JM109, JM101, JM107, Y1088, Y1089, Y1090, Y1090(ZZ), DM1, PH10B, DH11S, DH125, RR1, TB1 and SURE, Bacillus subtilis, Agrobacterium tumefaciens, Bacillus megaterium; and eukaryotic cells such as Pichia pastoris, Chlamydomonas reinhardtii, Cryptococcus neoformans, Neurospora crassa, Podospora anserina, Saccharomyces cerevisiae, Saccharomyces pombe, Uncinula necator, cultured insect cells, cultured chicken fibroblasts, cultured hamster cells, cultured human cells such as HT1080, MCF7, 143B and cultured mouse cells such as EL4 and NIH3T3 cells.

The present invention also provides a purified sodium/iodide symporter and analogues thereof and includes sodium/iodide symporter isolated from thyroid tissue obtained from a subject or sodium/iodide recombinantly produced. As used herein "analogues" mean amino acid-substituted derivatives of the sodium/iodide symporter which have ability to catalyze the sodium-dependent transport of iodide into a cell.

The present invention also provides for antibodies immunoreactive with sodium/iodide symporter and/or analogues thereof as well as antibodies immunoreactive with non-functional sodium/iodide symporter, i.e., sodium/iodide symporter which is inactive or exhibits only reduced iodine transport activity in vivo. The non-functional sodium/iodide symporter recognized by the antibodies of the present invention may result from one or more mutations in the nucleic acid encoding sodium/iodide symporter or from one or more deficiencies in the cell's protein synthesis and maturation pathways resulting in sodium/iodide symporter which is non-functional due, for example, to altered

secondary or tertiary structure. The antibodies of the present invention may be monoclonal or polyclonal and are produced by techniques well known to those skilled in the art, e.g., polyclonal antibody can be produced by immunizing a rabbit, mouse, or rat with purified sodium/iodide symporter and monoclonal antibody may be produced by removing the spleen from the immunized rabbit, mouse or rat and fusing the spleen cells with myeloma cells to form a hybridoma which, when grown in culture, will produce a monoclonal antibody. Labeling of the antibodies of the present invention may be accomplished by standard techniques using one of the variety of different chemiluminescent and radioactive labels known in the art. The antibodies of the present invention may also be incorporated into kits which include an appropriate labeling system, buffers and other necessary reagents for use in a variety of detection and diagnostic applications.

The present invention also provides nucleic acid probes and mixtures thereof which are hybridizable to sodium/iodide symporter. Nucleic acid probes may be prepared by a variety of techniques known to those skilled in the art such as PCR and restriction enzyme digestion of sodium/iodide nucleic acid or by automated synthesis of oligonucleotides whose sequence correspond to selected portions of the nucleotide sequence of the sodium/iodide symporter nucleic acid using commercially available oligonucleotide synthesizers such as the Applied Biosystems Model 392 DNA/RNA synthesizer. The nucleic acid probes of the present invention may also be prepared so that they contain one or more point, insertion or deletion mutations or a combination thereof to correspond to mutations of the sodium/iodide symporter gene.

The nucleic acid probes of the present invention may be DNA or RNA and may vary in length from about 8 nucleotides to the entire length of the sodium/iodide symporter nucleic acid. Labeling of the nucleic acid

probes may be accomplished using one of a number of methods known in the art, e.g., PCR, nick translation, end labeling, fill-in end labeling, polynucloetide kinase exchange reaction, random priming, or SP6 polymerase (for riboprobe preparation) and one of a variety of labels, e.g., radioactive labels such as ^{35}S, ^{32}P or ^{3}H or nonradioactive labels such as biotin, fluorescein (FITC), acridine, cholesterol or carboxy-X-rhodamine (ROX). Combinations of two or more nucleic probes corresponding to different or overlapping regions of the sodium/iodide symporter nucleic acid may also be included in kits for use in a variety of detection and diagnostic applications.

The present invention also provides methods for diagnosing a thyroid disorder in a subject which is associated with reduced or undetectable iodide transport activity in the subject's thyroid cells. As used herein, "subject" may be a fetus, newborn, infant, child or adult. Reduced or undetectable levels of iodide transport activity in thyroid cells is associated with a number of diseases such as hypothyroidism, hyperthyroidism, thyroid cancer and congenital lack of an iodide transport system. Alterations in iodide transport activity may be due to a decrease in the concentration of sodium/iodide symporter in the thyroid cell or due to the presence of non-functional sodium/iodide symporter.

Thyroid disorders resulting from a decreased concentration of sodium/iodide symporter may be diagnosed by nucleic acid hybridization and/or immunological techniques well known in the art. For example, nucleic acid hybridization studies of mRNA extracted from thyroid cells by sodium/iodide-specific nucleic acid probes can be used to determine the concentration of sodium/iodide symporter mRNA present in the cell and the concentration thus obtained, compared to the value obtained for thyroid cells which exhibit a normal level of iodide transport activity. Isolation of RNA from cells may be accomplished by a number of techniques, e.g., whole cell RNA can be

extracted using guanidine thiocyanate; cytoplasmic RNA may be prepared by using phenol extraction methods; and polyadenylated RNA may be selected using oligo-dT cellulose. Alternatively, the concentration of sodium/iodide symporter in the cell may be determined from binding studies using antibody immunoreactive with sodium/iodide symporter.

Thyroid disorders resulting from mutations in the nucleic acid encoding sodium/iodide symporter may be detected by one of a number of methods, e.g., hybridization analysis of nucleic acid extracted from a sample of tissue or cells from a subject's thyroid using nucleic acid probes designed to detect the presence of mutations in the nucleic acid encoding sodium/iodide symporter. Alternatively, the thyroid disorder may be detected using antibody immunoreactive with non-functional sodium/iodide symporter and standard immunological detection techniques such as Western blotting.

The present invention also provides a method for treating a thyroid disorder in a subject which is caused by mutation(s) in the sodium/iodide nucleic acid which encode a non-functional sodium/iodide symporter. The method of the present invention comprises introducing nucleic acid encoding a functional sodium/iodide symporter into substantially all the cells of a subject's thyroid. The nucleic acid encoding the sodium/iodide symporter may be RNA or DNA and may be introduced into the subject's thyroid cells by any one of a number of techniques, e.g., infecting cells with a recombinant viral vector derived from a DNA or RNA virus or a retrovirus which contains nucleic acid encoding a functional sodium/iodide symporter. In the method of the present invention, nucleic acid encoding functional sodium/iodide symporter is introduced into the subject's thyroid cells such that the cells express functional sodium/iodide symporter and are capable of iodide transport activity sufficient to treat the subject's thyroid disorder.

The present invention also provides a recombinant viral vector for use in such applications as gene therapy. The recombinant viral vector of the present invention comprises at least that portion of a viral genome which enables the virus to infect a target cell. In the recombinant viral vector of the present invention the viral nucleic acid sequences are operably linked to nucleic acid encoding sodium/iodide symporter such that, upon introduction of the recombinant viral vector into the target cell, sodium/iodide symporter is expressed in the target cell and the target cell is capable of transporting iodide. The recombinant viral vector of the present invention may further comprise specifically engineered promoter-enhancer sequences to achieve sodium/iodide symporter expression localized to only the target cell and/or a high level of sodium/iodide symporter expression in the target cell. Promoter-enhancer sequences include but are not limited to herpes promoter IE 4/5, cytomegalovirus-1 promoter and the Rous sarcoma virus long terminal repeat.

Recombinant viral vectors suitable for gene therapy include but are not limited to vectors derived from the genomes of viruses such as HSV, adenovirus, adeno-associated virus, Semiliki Forest virus, cytomegalovirus and vaccinia virus. Examples of suitable viral vectors for the introduction of sodium/iodide symporter into target cells are HSVprPUC, pZeoSV (Invitrogen), pcDNA3 (Invitrogen), pRc/CMV (Invitrogen), pRc/RSV (Invitrogen), pSVT7 (Life Technologies), p91023(B) (Life Technologies), pMB1 (Clontech), pEUK-C1 (Clontech), pMAMneo (Clontech), pMAMneo-CAT (Clontech), pMAMneo-LUC (Clontech), pDR2 (Clontech), pADα (Clontech), pADß (Clontech), pCMVß (Clontech) and pGFP (Clontech). The choice of recombinant viral vector will be determined by the characteristics of the target cell population, e.g., recombinant viral vectors such as those based on adenovirus and herpes virus are suited to target cells

which have minimal mitotic activity because these vectors do not integrate into th genome; in contrast, the use of a recombinant viral vector which integrates into the genome such as a vector based on adeno-associated virus is preferred in mitotically active cell populations. Techniques for preparing stocks of recombinant viral vectors are well known in the art and may, for example, require co-infection of a "packaging cell line" with the recombinant viral vector and a helper virus. The helper virus and packaging cell line contain all the genes necessary to replicate the viral vector and package it into virions.

The present invention also provides a method for selectively ablating thyroid or non-thyroid tissue in a subject and may be used to excise inoperable tumors or as a non-invasive alternative to surgery. The method of the present invention involves the introduction of nucleic acid encoding sodium/iodide symporter into substantially all of the cells of a target tissue such that those cells express sodium/iodide symporter and are capable of iodide transport. Methods for introducing sodium/iodide symporter into cells are discussed above. Ablation of the target tissue is accomplished by treating the subject with a radioactive isotope of iodine following introduction of the sodium/iodide symporter. The use of radioisotopes of iodine is well known in the art, e.g., ^{131}I is the most commonly used isotope in clinical practice. The radioisotope of iodine to be used, its dosage and the means of delivery will be dependent upon a number of factors, e.g., variability in intersubject pharmacokinetic parameters and the type of target tissue, but would be apparent to one skilled in the art. In particular, the method of the present invention may be used to treat thyroid cancer in a subject. The majority of thyroid cancers display markedly reduced iodide transport activity making them resistant to radiotherapy with radioisotopes of iodine. The method of the present invention, by

endowing iodide transport activity on the cells of the thyroid tumor thereby restores the use of radioactive iodide as a potentially viable treatment modality.

The present invention also provides a method for identifying an iodide transport protein from non-thyroid tissue including but not limited to the salivary gland, gastric mucosa, lactating mammary gland, choroid plexus and the ciliary body of the eye. Iodide transport proteins from non-thyroid tissues may be identified by a number of techniques known in the art, e.g., screening a cDNA library prepared from the tissue of interest using nucleic acid encoding all or a portion of sodium/iodide symporter as a probe and assessing the ability of cDNA clones thus identified to endow iodide transport activity in COS cells or Xenopus leavis oocytes. Labeling of nucleic acid probes has been discussed previously and methods for introducing nucleic acid into COS cells or Xenopus leavis oocytes and functional screening are discussed in the Experimental Details Section which follows.

Finally, the method of the present invention provides a non-human, transgenic animal model for a thyroid disorder. The animal model of the present invention comprises a non-human, transgenic animal having nucleic acid encoding mutated sodium/iodide symporter incorporated into thyroid tissue. The mutated nucleic acid encoding sodium/iodide symporter may consist of nucleic acid isolated from the thyroid cells of subjects suffering from thyroid disorders associated with non-functional sodium/iodide symporter.

Nucleic acid encoding mutated sodium/iodide symporter may be integrated into the germ line of a non-human animal such as a mouse, rat, goat, sheep or other species in order to obtain a transgenic animal. Expression of the incorporated nucleic acid may be r stricted to the thyroid tissue in the transgenic animal by the utilization of tissue-specific promoters. Methods

of making transgenic animals are well known in the art. For example, DNA encoding mutated sodium/iodide symporter can be inserted into the genome of a replication-defective virus such as HSV, or a retrovirus or transposon and the resultant construct injected into embryonic stem cells. Transgenic animals may also be made by injecting DNA encoding mutated sodium/iodide symporter into the male pronucleus of a fertilized egg of a nonhuman animal, transplanting the "transgenic embryo" into a pseudopregnant female and then analyzing offspring for the presence of the injected DNA in their genome. Other methods of producing transgenic mice would be apparent to one skilled in the art.

The present invention is described in the following Experimental Details Section which is set forth to aid in the understanding of the invention, and should not be construed to limit in any way the invention as defined in the claims which follow thereafter.

Experimental Details Section

1. Materials and Methods

A. Isolation of cDNA Encoding Sodium/Iodide Symporter

A directional cDNA library was prepared in the pSPORT (BRL) vector from poly A^+ RNA isolated from FRTL-5 cells. cRNAs were synthesized in vitro using T7 RNA polymerase in the presence of m^7GpppG from pools of cDNA clones. Transcripts were injected into oocytes and oocytes were assayed for perchlorate-sensitive Na^+/I^- symport activity as described in Weiss, et al., supra. Clones from one positive pool were subdivided and assayed successively until a single sodium/iodide symporter cDNA clone was isolated. COS-7 cells were grown in 6-well plates (37°C, 5% CO_2) in high glucose Dulbecco's modified Eagles medium (DMEM) supplemented with 10% fetal calf serum. Cells were transfected when they reached approximately 70% density. Cells were rinsed with serum-free medium and incubated with Opti-MEM1 (Gibco)

containing 0.5mg/ml DEAE-Dextran (MW $2x10^6$) and 0.66 μg/well column-purified (Qiagen) plasmid DNA for 30 min at 37°C, followed by a 3 hr incubation with 0.1 mg/ml chloroquine in DMEM. Iodide accumulation assays were conducted 48 h after transfection using previously described procedures (Kaminsky, et al., supra).

B. Sequence Analysis

Both strands of the sodium/iodide symporter cDNA were sequenced using Sequenase version 2.0 (U.S. Biochemical).

C. Northern Blot Analysis

Thirty μg of total RNA was size fractionated in a denaturing 0.66 M formaldehyde, 1.5% agarose gel (1x MOPS buffer), and transferred to a nylon membrane by overnight capillary blotting in 10X SSC. RNA quality was assessed by ethidium bromide staining. Nylon membranes were hybridized overnight at 42°C with [^{32}P]αdCTP labeled sodium/iodide symporter cDNA in 6x SSC, 5x Denhardt's reagent, 0.5% SDS, 100 μg/ml denatured salmon sperm DNA and 50% formamide. Membranes were washed twice for 30 min at room temperature with 2xSSC/0.1%SDS, and then for 15 min at 55°C in 0.1%SSC/0.1%SDS. Sodium/iodide symporter and cyclophillin cDNAs were ^{32}P-dCTP radiolabelled by random primed synthesis (Amersham).

II. Results

A. Isolation and Characterization of the Sodium/Iodide Symporter cDNA Clone

A rat thyroid cDNA library constructed in the pSPORT vector was size fractionated and the fraction containing inserts from 2.5-4.5kb was subjected to functional screening in oocytes. cRNAs synthesized in vitro from pools of cDNA clones from this fraction were microinjected into oocytes and assayed for perchlorate-sensitive sodium/iodide (Na^+/I^-) symport activity. As shown in Figure 1A, positive pools containing successively

fewer cDNA clones were assayed until a single positive clone was identified. Activity elicited by mRNA from FRTL-5 cells is also shown (lane 2). Control assays were carried out in oocytes microinjected with water (lane-1). Na^+-dependence was ascertained by using choline in place of Na^+, and perchlorate sensitivity was tested by conducting assays in the presence of both Na^+ and perchlorate. In all cases, activity measured in the absence of Na^+ or in the presence of perchlorate was virtually indistinguishable from background. At the bottom of Figure 1A the number of clones in each pool tested is shown above the number of days (in parentheses) elapsed after microinjection when Na^+/I^- symport activity was first detected. A clear correspondence is apparent between the decreasing number of clones in the pools, the rising magnitude of the activity elicited, and the shortening of the latency period for appearance of the signal after injection.

Maximal expression of Na^+/I^- symport activity was observed 2-3 days after injection. The time course of iodide accumulation was assessed 60 h after oocytes were injected with the transcript from the sodium/iodide symporter cDNA clone. As shown in Figure 1B, iodide accumulation reached nearly 800 pmol of iodide/oocyte at the approximately 90 min saturation point. Given that the iodide concentration in the transport solution was 50 μM and the functional volume is 0.5μl/oocyte, the generated iodide concentration gradient was >30 fold, i.e. virtually identical to that observed in the thyroid gland in vivo (Carrasco, Biochim. Biophys. Acta., supra). This signal corresponds to a >700-fold increase in perchlorate-sensitive Na^+/I^- symport activity over background. The kinetic analysis of Na^+/I^- symport activity in oocytes microinjected with the transcript from the symporter cDNA clone is depicted in Figure 1C. Initial velocity rates of Na^+/I^- symport activity were determined at the 2 min time points at various iodide concentrations (1.25 to 250 μM).

Na^+/I^- symport rates were found to display saturation kinetics (Figure 1C). The double reciprocal plot of the same data is shown in the inset. The apparent K_m for iodide was 36 μM, a value consistent with the range of values reported for FRTL-5 cells (Vilijn and Carrasco, J. Biol. Chem. 264:11901-11903 (1989); Carrasco, supra). As shown in Figure 1D, COS cells transfected with the sodium/iodide symporter cDNA clone exhibited perchlorate-sensitive Na^+/I^- symport activity, in contrast to control non-transfected cells or cells transfected with the same plasmid containing an irrelevant insert, neither of which displayed symport activity. These results provide unequivocal proof that the product of the sodium/iodide symporter cDNA clone is sufficient to elicit perchlorate-sensitive Na^+/I^- symport activity in both oocytes and mammalian cells, and suggest that the symporter functions as a single subunit or as an oligomer of identical subunits.

A nucleic acid contained in the vector pSPORT was identified as containing the entire coding region of the rat sodium/iodide symporter and was designated pNIS. pNIS was deposited under the terms of the Budapest Treaty with the American Tissue Culture Collection (ATCC) on February 1, 1996 under Accession No. _____.

B. Primary Sequence and Predicted Secondary Structure of the Sodium/Iodide Symporter Molecule

The complete nucleotide sequence of the cloned sodium/iodide symporter cDNA and the deduced amino acid sequence are presented in Figure 2. The nucleotide sequence of the sodium/iodide symporter clone indicates that the insert is 2,839 base pairs in length, with a predicted open reading frame of 1854 nucleotides, a 5′ untranslated region of 109 nucleotides and a 3′ untranslated region of 876 nucleotides. Within the 3′ untranslated region, a potential poly A signal was identified at position 2795. The putative initiation

codon ATG contains a purine at position 113 and thus repres nts a reasonable Kozak consensus sequence (Kozak, J. Biol. Chem. 266:19867-19870 (1991)). Beginning with Met at position 1, a long open reading frame codes for a protein of 618 amino acids (relative molecular mass 65,196). The hydropathic profile (Figure 3A) and secondary structure predictions (Kyte and Doolittle, supra; Chou and Fasman, Biochemistry 13:222-245 (1974)) (not shown) of the protein suggest that the cloned cDNA encodes for an intrinsic membrane protein with 12 putative transmembrane domains (Figure 3B). In Figure 3B the NH_2 terminus has been placed on the cytoplasmic side, given the absence of a signal sequence. The COOH terminus, which has also been predicted to be on the cytoplasmic side, contains a large hydrophilic region of approximately 70 amino acids within which the only potential cAMP-dependent PKA phosphorylation domain of the molecule is found (positions 549-552). Three potential Asn-glycosylation sites were identified in the deduced amino acid sequence at positions 225, 485, and 497. The last two are located in the 12th hydrophilic sequence, a domain predicted to be on the extracellular face of the membrane. The length of the 12 transmembrane domains in the model ranges from 20 to 28 amino acid residues, except helix V, which contains 18 residues. Only three charged residues are predicted to lie within transmembrane domains, namely Asp 16 in helix I, Glu 79 in helix II, and Arg 208 in Helix VI. All three charged residues are located close to the cytoplasmic side of the corresponding domain rather than towards its center. Out of a total of 8 Trp residues found in the membrane, six are located near the extremes of transmembrane domains. The lengths of transmembrane domains and location of Trp residues proposed in the sodium/iodide symporter secondary structure model are similar to those found in the R.viridis photoreaction center crystal structure (Deisenhofer and Michel, EMBO J. 8:2149-2170 (1989)). Four Leu residues (positions 199,

206, 213 and 220) appear to comprise a Leu zipper motif. This motif, which has been proposed to play a role in the oligomerization of subunits in the membrane, has been conserved in all cloned neurotransmitter transporters (Surratt, et al., Curr.Opin Nephrol.Hypertens 2:744-760 (1993)). A comparison of the predicted amino acid sequence of sodium/iodide symporter which those of other cloned Na^+-dependent cotransporters in available databases (Swissprot.) revealed the highest degree of homology (24.6% amino acid identity) with the human Na^+/glucose cotransporter (Hediger, et al., Proc.Natl.Acad.Sci.USA 86:5748-5752 (1989)). Sodium/iodide symporter falls alongside other anion transporters in a dendrogram representing a cluster analysis of Na^+-dependent cotransporters on the basis of their amino acid sequences (Figure 4).

A 2.9 kb mRNA transcript that hybridizes with isolated sodium/iodide symporter cDNA clone was identified by Northern analysis (not shown) in both FRTL-5 cells and rat thyroid tissue, but not in such non-thyroid tissues as rat brain, intestine, heart, kidney and liver, thus suggesting that sodium/iodide symporter is primarily expressed in thyroid cells. As expected, the sodium/iodide symporter transcript was more readily detectable in the epithelial cell line than in thyroid tissue.

C. Identification of cDNA Encoding Human Sodium/Iodide Symporter

In order to identify the human sodium/iodide symporter, a cDNA library prepared from human thyroid will be screened using DNA encoding the rat sodium/iodide symporter as a probe. cRNAs will be synthesized in vitro from cDNA clones identified as potentially containing human sodium/iodide symporter-specific sequences in vitro using T7 RNA polymerase in the presence of m^7GpppG. Transcripts will be injected into oocytes and oocytes then assayed for perchlorate-sensitive Na^+/I^- symport activity.

III. Conclusion

In summary, the rat sodium/iodide symporter cDNA clone has been isolated and a secondary structure model for the sodium/iodide symporter molecule designed. It has been shown that the product of the sodium/iodide symporter cDNA clone is sufficient to elicit Na^+/I^- symport activity in both oocytes and mammalian cells, suggesting that sodium/iodide symporter probably functions as a single subunit. The data presented here establish that rat sodium/iodide symporter is a 618 amino acid (relative molecular mass 65,196) Na^+-dependent intrinsic membrane protein with 12 putative transmembrane α-helix domains. The role of sodium/iodide symporter as the mediator of iodide accumulation is consistent with its placing alongside other Na^+-dependent anion transporters on the basis of clustering relationships between its deduced amino acid sequence and the deduced sequences of other transporters. Given that some of the residues important for function in several membrane transporters are charged amino acids located in putative transmembrane domains (Carrasco, et al. Biochemistry 25:4486-4488 (1986); Zhang, et al., J. Biol. Chem. 269:19573-19577 (1994); Pantanowitz, et al., J. Biol. Chem. 268:3222-3225 (1993)), it is suggested that one or more of the three such residues in sodium/iodide symporter, namely Asp 16, Glu 79 and Arg 208, may play a role in Na^+/I^- symport activity. In Northern blot analyses using the sodium/iodide symporter cDNA clone as a probe it was observed that 2.9 kb transcript is present in FRTL-5 cells and in the thyroid gland but not in brain, intestine, heart, kidney or liver.

The cloning and characterization of sodium/iodide symporter constitutes the full molecular identification of the protein that mediates iodide accumulation in the thyroid, i. . the key initial step in thyroid hormogenesis. A corollary of the present

characterization of rat sodium/iodide symporter is that the pathophysiology of the thyroid iodide accumulating system can now be studied at the molecular level. Considering the high degree of homology among eukaryotic transport proteins from different species, it is likely that the human sodium/iodide symporter cDNA clone will be readily isolated in the near future. Hence, it will be possible to explore the expression of human sodium/iodide symporter in such thyroid pathological states as hyper and hypothyroidism, thyroid cancer, and congenital lack of the iodide transport system. The results reported here suggest that elucidation of the molecular mechanism of thyroidal active iodide accumulation and its regulation is within reach.

All publications mentioned hereinabove are hereby incorporated by reference in their entirety.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be appreciated by one skilled in the art from a reading of the disclosure that various changes in form and detail can be made without departing from the true scope of the invention in the appended claims.